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Abstract

Regenerative medicine is a diverse and rapidly evolving field, employing core expertise from biologists, engineers, and clinicians. Recently the field has made significant progress towards regenerating or replacing tissues lost to age, disease or injury. Current strategies include transplantation of adult or pluripotent stem cells to replace tissue or support tissue healing. Promising approaches for the future of regenerative medicine include stimulating endogenous stem cells for in situ repair, transplantation of organoids to repair minor tissue injury, and the use of interspecies chimerism to produce functional metabolic organs for transplantation. In our review we focus on these emerging strategies, paying particular attention to their current and prospective translational impacts and challenges.

Keywords	adult stem cells; pluripotent stem cells; organoids; chimeras; senolytics
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Bench to bedside: Current advances in regenerative medicine

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Abstract

Regenerative medicine is a diverse and rapidly evolving field, employing core expertise from biologists, engineers, and clinicians. Recently the field has made significant progress towards regenerating or replacing tissues lost to age, disease or injury. Current strategies include transplantation of adult or pluripotent stem cells to replace tissue or support tissue healing. Promising approaches for the future of regenerative medicine include stimulating endogenous stem cells for *in situ* repair, transplantation of organoids to repair minor tissue injury, and the use of interspecies chimerism to produce functional metabolic organs for transplantation. In our review we focus on these emerging strategies, paying particular attention to their current and prospective translational impacts and challenges.

Introduction

Transplantation of adult stem cells is a well-established technology to treat disease, with hematopoietic stem cell transplantation in wide spread clinical use for over 50 years. Nevertheless, this technology continues to find new clinical applications. For example, recent results from an international clinical trial showed that high-dose immunosuppressive therapy and subsequent autologous hematopoietic stem cell (HSC) transplantation is effective in inducing long-term sustained remissions of active relapsing-remitting multiple sclerosis [NCT00273364] [1]. In this trial only 6% of patients treated with HSCs relapsed, compared to 60% in the untreated control group. Similarly, autologous cultured epidermis transplantation is a long-established approach. Notable progress has recently been made in the treatment of

skin blistering disorders by gene correction of epidermal cells, followed by their expansion in culture and subsequent transplantation [2]**. In a recent world first, this graft technique was used to completely replace the skin of a child with epidermolysis bullosa (EB).

Allogeneic MSC transplantation has also been widely used in patients to treat a range of conditions [3]. However, evidence of efficacy has been lacking in many cases. Recent studies of patients with graft-versus-host disease (GvHD) suggest that successful treatment is determined by the state of the patient's immune system rather than by the state of the infused MSC. Galleu *et al.* (2017) found that patients who had high cytotoxic T cell activity against MSCs responded to MSC infusion, whereas those with low activity did not [4]. After infusion, host phagocytes engulfed apoptotic MSCs and produced indoleamine 2,3-dioxygenase, which was necessary for the immunosuppressive effect of MSCs. This suggests that patients should be stratified for MSC treatment according to their cytotoxic T cell activity, or, alternatively that patients could be treated with apoptotic MSCs.

While adult stem cell therapies continue to find new and exciting clinical applications, adult stem cells can only give rise to a limited number of cell types and can be hard to scale. This has driven the development of many next-generation regenerative strategies. The new approaches include methods to promote endogenous tissue repair, through the use of pharmacological bio-scaffolds [5], in vivo cell reprogramming [6], and senolytics [7], methods to produce novel autologous cell therapies using induced pluripotent stem cells (iPSCs) [8]*, and techniques aimed at engineering functional human tissues and organs through in vitro tissue engineering [9]** and interspecies chimerism [10]**. In this review we will focus on these emerging strategies in the field of regenerative medicine and discuss the technical and translational challenges they pose.

In situ regeneration

An attractive strategy for regenerative medicine is to target endogenous repair mechanisms in resident cell populations. A promising approach for this is through the recruitment of tissue-resident stem populations following trauma. Implanted biological scaffolds (bio-scaffolds) provide structural support for functional tissue regrowth and facilitate localised delivery of pro-regenerative agents [11]. A recent advance has been the use of a clinically-approved degradable collagen sponge seeded with a GSK3 inhibitor to promote mesenchymal stem cell (MSC)-based dentine regeneration in a rodent model. While this represents a new approach to tooth repair [5], significant concerns are the off-target effects of broad stimulation of pro-regenerative pathways and the possibility that continued

activation of somatic stem cells might lead to their depletion and loss of regenerative capacity.

An alternative *in situ* approach for regenerative medicine is reprogramming of terminally differentiated cells. One current strategy for *in vivo* reprogramming involves enforcing expression of key regulatory transcription factors (TFs) to convert cells to an alternative mature state [6]. This strategy can be employed to generate functional cells to replace those lost through disease. Insulin positive pancreatic β-like cells have been successfully generated in mice from pancreatic exocrine [12], hepatic [13] and gastrointestinal [14] cells following virus-mediated delivery or transgenic expression of a combination of pancreatic TFs. This partially alleviates hyperglycaemia in diabetic murine models. Alternatively pathological cells can be reprogrammed to ameliorate disease, as with the conversion of myofibroblasts to hepatic-like cells in induced murine models of liver damage, through adenovirus-mediated induction of a combination of hepatic TFs [15,16]. Nevertheless, the efficiency of reprogramming cells *in vivo* is generally low, which may contribute to the limited translation of this approach into the clinic.

Cellular senescence has recently been characterised to promote *in vivo* reprogramming. Senescent cells have permanently withdrawn from the cell cycle and adopt a senescence-associated secretory phenotype (SASP) that is characterised by the secretion of a range of factors, including IL-6 [17]. Treatment of animals with an anti-IL-6 antibody decreases the expression of SASP components and significantly reduces reprogramming in mice using the Yamanaka factors Oct4, Sox2, Klf4, and c-Myc (OSKM) [18]*. Conversely, transient treatment with recombinant IL-6 increases the number of *in vivo* reprogrammed cells [18]*. In contrast, chronic exposure to SASP components is thought to hinder native regeneration in multiple tissues [19]. Pharmacological agents which induce apoptosis in senescent cells (senolytics) [7] have been shown to positively influence regenerative capacity in several models of age-related disease [20–23]. Naturally aged mice treated with senolytics have significantly improved hematopoietic stem cell serial repopulation capacity [20], reduction in age-associated myeloid skewing [20] and increased renal function and hair follicle density [22]. A proposed model to explain the dual role of senescence in plasticity and regeneration is the length of exposure to SASP components, whereby short-term exposure is beneficial, but prolonged exposure induces further senescence and a regenerative block [24].

Pluripotent stem cell therapies

Pluripotent stem cells (PSCs) have the capacity for unlimited self-renewal and the ability to differentiate into any cell type. This makes them an attractive alternative to lineage-restricted adult stem cells, and particularly amenable to cell replacement therapies involving difficult to obtain cell types. Delivery of PSCs or their differentiated progeny into damaged tissues with low regenerative capacity may aid tissue repair by replacing lost cells or providing trophic support [25].

Due to its accessibility, immunoprivileged nature and high clinical need, the eye is an attractive target for cell replacement therapies. The first evidence of long-term safety, graft survival and efficacy of transplanted pluripotent stem cells was demonstrated in 2016: a suspension of retinal pigmented epithelial (RPE) cells derived from embryonic stem cells (ESC-RPE) was injected into the eyes of nine patients with age-related macular degeneration (AMD), resulting in sustained visual improvement in approximately half of treated individuals up to 37 months [26]. Culture of RPE on biological scaffolds, and transplantation as a more physiologically-representative 'sheet', shows optimised differentiation, polarisation, engraftment, viability and efficacy when compared to cell suspensions [27]. In a world-first induced pluripotent stem cell (iPSC) trial, integration-free autologous iPSC-RPE sheets were grown on collagen and successfully engrafted into one patient with AMD (UMIN000011929). However, visual improvements have not been reported after one year [8]*. Recently, ESC-RPE sheets have been manufactured on decellularized amniotic membrane[28] and a synthetic vitronectin-coated polyester membrane [29]**. Successful transplantation of this 'synthetic' ESC-RPE patch in two patients has resulted in significant improvements in visual acuity 12 months after surgery with only local immunosuppression (NCT01691261) [29]**.

Recently, integration-free iPSC-derived neural stem/progenitor cells (iNSPCs) generated from patient urine samples were shown to successfully engraft and differentiate in a mouse model of spinal cord injury (SCI) [30]. Transplantation of iPSC-derived dopaminergic neurons (iDAs) was demonstrated to ameliorate locomotor symptoms of Parkinson's disease (PD) in rodents [31,32] and primates [33], and clinical trials are expected to begin soon [34]. Indeed, a clinical trial involving NSPCs derived from chemically-activated 'parthenogenetic' embryos to treat PD is currently underway (NCT02452723), following improved behavioural recovery after intracerebral transplant in Parkinsonian monkeys [35]*.

Epidurally transplanted iNSPCs have been used to enhance functional recovery after stroke in rats by paracrine activation of Notch1 signalling in endogenous progenitors [36]. A permissive microenvironment promoting engraftment, survival and maturation can also be achieved by exogenous addition of growth factors [37,38] or genetic engineering of

transplanted cells to overexpress neurotrophic factors such as SDF-1α [39], Nurr1 and Foxa2 [40].

In conclusion, sophisticated methods for PSC derivation and transplantation have advanced basic research, but extensive preclinical analysis of all cell therapies with respect to cell origin (embryo, foetus, iPSC), tumorigenicity, potency, administration strategy (suspension, monolayer, encapsulation [41]) and mode of action must be performed to enable safe integration into the clinic [42]*. Clinical trials must begin to recruit more participants to enable robust statistical analysis of each cell therapy. Rapid delivery of thoroughly regulated personalised therapies within a critical treatment window may not be feasible for many acute diseases, unless banks of ESCs or iPSC are developed. Finally, these therapies are often not suitable for polygenic or systemic diseases affecting more complex structures.

3D-cultured organoids

Advances in 3D tissue engineering allow for the generation of 'miniature organs' (organoids) *in vitro* [43]. Organoids are self-renewing, self-organising 3D cell clusters that contain organ-specific cell types and mimic organ function and morphology. While organoids can be obtained from multicellular tissue samples, such as intestinal crypts [44], they can also be grown and expanded from pluripotent stem cells [43]. Although organoids have been successfully cultured to simulate organs such as kidney [45], liver [46], small intestine [43] and brain [47]*, until recently they have mostly found applications in developmental biology research and disease modelling [48].

Organoids are commonly cultured in Matrigel, an undefined tumour-derived extracellular matrix (ECM), and this hampers their potential use in the clinic due to risks of immunogen and pathogen transfer [49]*. To circumvent this issue, Cruz-Acuna *et al.* substituted Matrigel with a fully defined synthetic hydrogel based on a four-armed, maleimide-terminated poly(ethylene glycol) (PEG-4MAL) macromer as a matrix for organoid culture [9]**. Human intestinal organoids (HIOs) grown in this matrix exhibited morphology and proliferation similar to those of HIOs grown in Matrigel. Moreover, when implanted under the kidney capsule of immunocompromised NSG mice, HIOs generated in PEG-4MAL successfully developed into mature intestinal tissue, exhibiting markers for intestinal epithelial, enteroendocrine, goblet and tuft cells, all found in the normal intestine. To further prove the feasibility of using HIOs clinically, Cruz-Acuna *et al.* injected HIOs at the site of mechanically induced mucosal wounds in the mouse colon with PEG-4MAL hydrogel as an injection vehicle, resulting in organoid engraftment and improved wound repair compared to controls

[9]** (Figure 2). This study shows that not only do intestinal organoids have clinical potential in the field of tissue repair, but also that fully defined, synthetic hydrogels can be used both as a culture matrix and as an injection vehicle for organoids, overcoming the obstacle presented by Matrigel-based organoid culture.

While attempts are being made to improve organoid technology so that it can be employed in the regeneration of other tissues, such as liver [46] and kidney [45], the long culture time and the lack of organ-specific macro-architecture and vascularisation limit the clinical applications of organoids to the repair of small defects, or of defects in organs with a relatively simple structure, such as the intestine. Therefore, a more ambitious tissue engineering approach may be necessary for the treatment of large defects in organs with complex architectures.

Interspecies chimeric organs

An innovative approach to treat severe tissue defects while also addressing the organ shortage crisis is the generation of whole organs via interspecies chimeras. Host animals can potentially be engineered to grow humanised organs suitable for transplantation, in a process called interspecies chimerism. Recent studies in rodents [10]** have demonstrated how, while technically feasible, this strategy would be clinically challenging.

Through genetic manipulation, it is possible to create a developmental niche in a host animal that can be populated by PSCs from a donor animal, generating a chimeric organ consisting mostly of donor cells. Cells are commonly injected at the blastocyst stage in an approach known as blastocyst complementation [50]. A recent proof-of-concept study by Yamaguchi *et al*. [10]** has demonstrated successful generation of mouse pancreata in Pdx-1-deficient rats using this technique. Islets from these rat chimeras were transplanted into mice with streptozotocin-induced diabetes, normalising blood glucose levels in the absence of immunosuppression (Figure 3).

Rat-mouse chimeric nephrons have also been generated following removal of nephron progenitor cells (NPCs) from the post-implantation rat foetus with a time-specific cell elimination strategy, creating a niche for injected mouse NPCs [51]. This establishes the possibility of generating organs with more complex developmental controls than the pancreas but is still far removed from whole kidney organogenesis.

The success of rat-mouse chimeras [52,53] has provided a foundation for generating human organs in a host species. Pigs serve as a good candidate animal due to their similar organ

size, physiology and anatomy. However, fluorescently labelled human PSCs (hPSC) injected into pig blastocysts show inefficient contribution to chimera formation [54]. An intermediate hPSC shows improved contribution and differentiation into several cell types post-implantation but contribution from all hPSCs is still much lower than that observed in rat-mouse chimeras. This may be due to the larger degree of evolutionary distance between humans and pigs. Future research might consider the use of non-human primates which are evolutionarily closer to humans.

Engraftment and survival of donor cells in chimeras can be improved by matching the developmental timing of cells to the host. Despite the large evolutionary distance, Cohen *et al*. [55] were able to generate mouse-human neural crest chimeras by injecting human neural crest cells (NCCs) into post-gastrulation mouse embryos at the stage when NCCs delaminate from the neuroepithelium. Donor cells survived, migrated along normal migration routes and contributed to functional pigment cells. Inhibiting apoptosis can improve integration of cells in later stages of development [56]. This may help prevent donor cells from contributing to other parts of the host, addressing a serious ethical concern [57]. Despite exciting progress, we are still a long way from generating human organs for transplantation, but chimeric animals may soon enable the study of human organ generation *in vivo*.

Concluding remarks

The field of regenerative medicine is advancing at an unprecedented rate. Adult stem cell transplantation, whilst well established in the clinic for some diseases, is not a viable solution for many other more localised disorders that may require specialised cell replacement. Pluripotent stem cells that can give rise to theoretically any cell type offer a promising alternative transplantation approach. Streamlining the generation of clinical-grade cell lines and reducing the tumourigenic risk associated with stem cell therapy are priorities for progression to wide-spread clinical use. Novel approaches targeting cell populations in situ are an exciting area of research, which could circumvent immunogenic risk associated with exogenous tissue transplantation. Considerations regarding target specific effects and long-term depletion of resident stem cell populations would need to be addressed for the advancement of this approach. Finally, more ambitious but less clinically advanced approaches aim to generate human organs *in vitro* or through interspecies chimerism. The ability to culture organs would have a considerable impact, alleviating the need for donor tissue. Key challenges include generating *in vitro* organoids with sufficiently complex vascularised networks and 3D architectures, alongside niche optimisation to facilitate

effective human interspecies chimerism. Future developments in the field of regenerative medicine will not only translate current strategies to the clinic, but also drive novel and complementary strategies to treat a broad range of diseases.

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Figure legends

Figure 1. Current strategies in regenerative medicine. Somatic stem cells can be targeted *in situ*, through the application of bioscaffolds seeded with pro-regenerative agents, to activate endogenous repair mechanisms [5]. Alternatively, tissue resident cells can be reprogrammed from a pathophysiological state [15,16] or to an alternative functional state [14,58] to alleviate disease. Adult, embryonic or induced pluripotent stem cells can also be transplanted to enhance regeneration. This may include *ex vivo* expansion and gene editing to correct pathogenic mutations. Transplantation of organoids shows promise as a strategy to improve regeneration [9]**. Finally, the generation of human-animal chimeras could facilitate the culture of entire human organs. Bioscaffolds, biological scaffolds.

Figure 2. Organoid culture and transplant for intestinal tissue regeneration. Human pluripotent stem cells can be induced to generate intestinal organoids that can then be expanded in PEG-4MAL hydrogel. The organoids can be delivered to the injury site using a hydrogel-based injection vehicle, resulting in ameliorated wound repair [9]**.

Figure 3. Schematic representation of blastocyst complementation to generate a functional mouse pancreas in a rat. Figure adapted from Yamaguchi *et al.* 2017 [10]**.

The authors declare no conflict of interest. FMW is on secondment as Executive Chair of the UK Medical Research Council.

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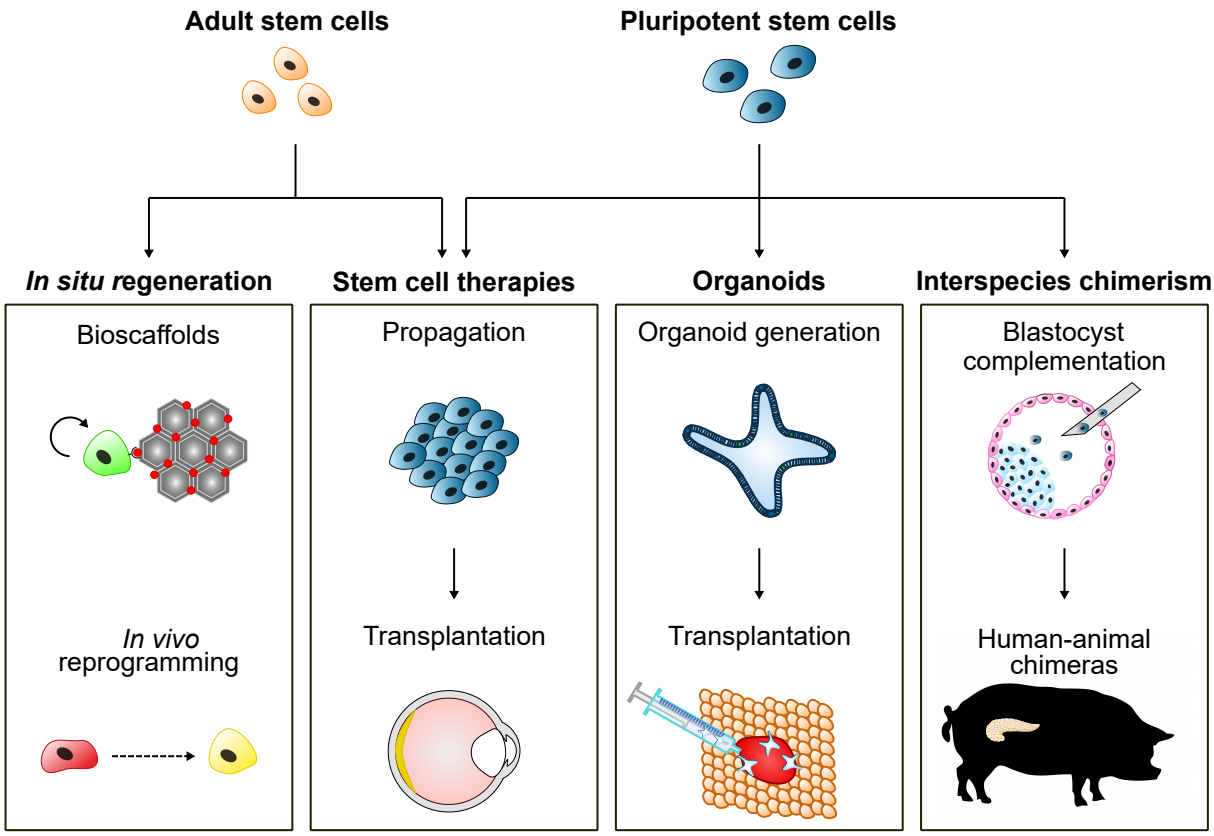
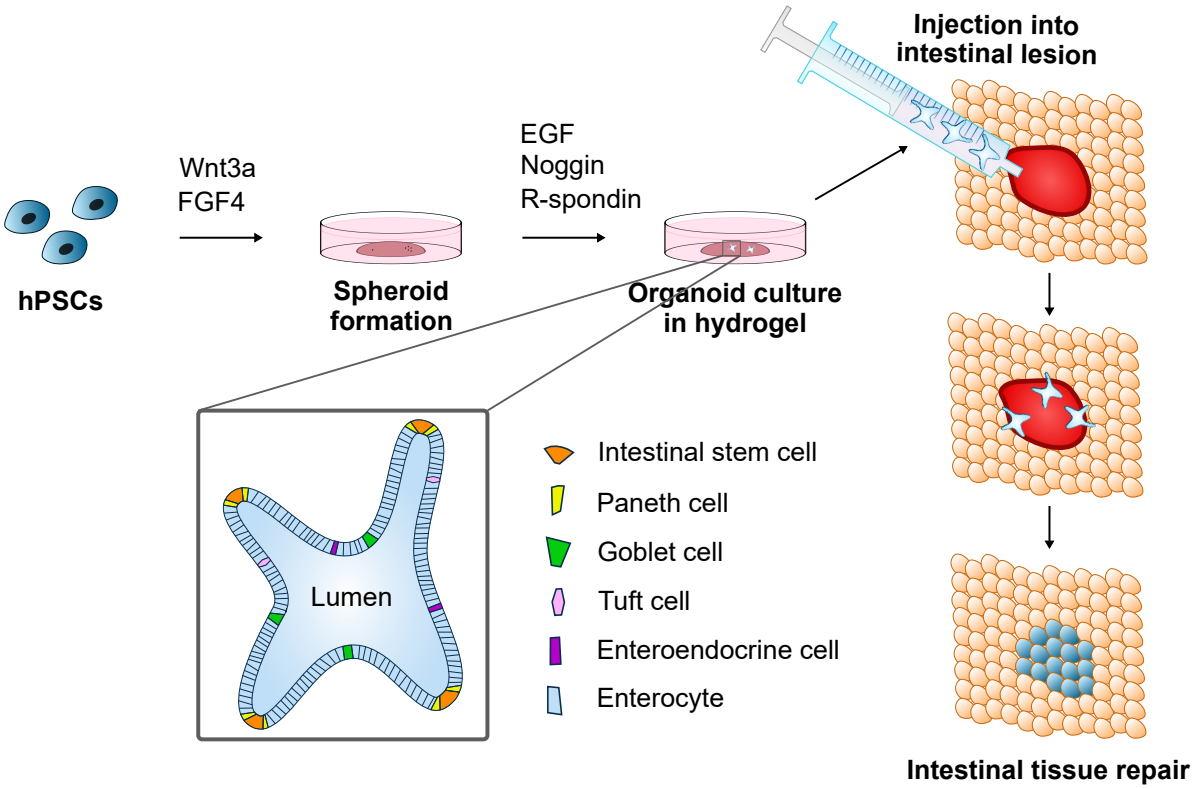
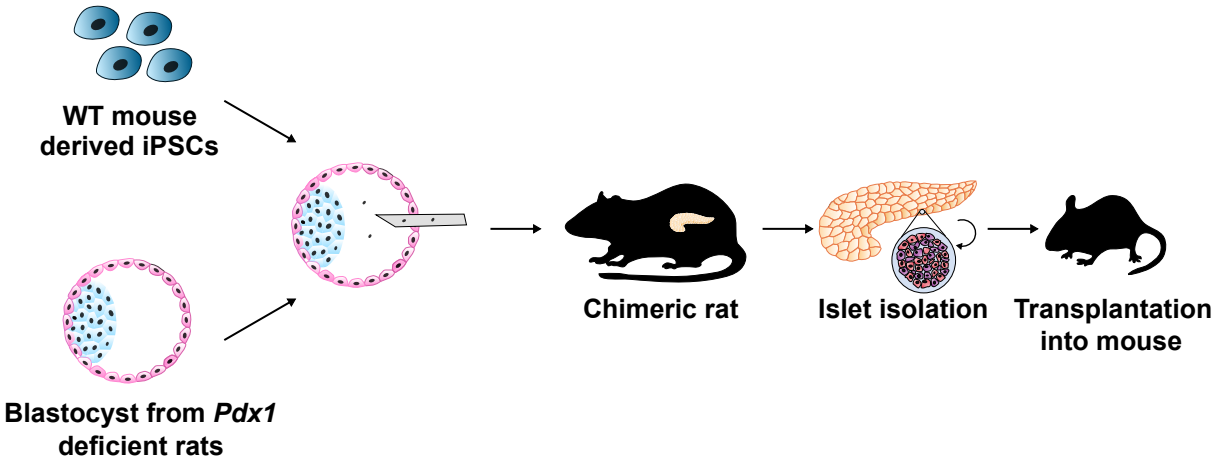

Adult stem cells
Pluripotent stem cells
In situ regeneration
Stem cell therapies
Organoids
Interspecies chimerism
Bioscaffolds
In vivo reprogramming
Propagation
Transplantation
Organoid generation
Transplantation
Blastocyst complementation
Human-animal chimeras


hPSCs
Wnt3a
FGF4
Spheroid formation
EGF
Noggin
R-spondin
Organoid culture in hydrogel
Injection into intestinal lesion
Lumen
Intestinal stem cell
Paneth cell
Goblet cell
Tuft cell
Enteroendocrine cell
Enterocyte
Intestinal tissue repair


WT mouse
derived iPSCs
Blastocyst from *Pdx1*
deficient rats
Chimeric rat
Islet isolation
Transplantation
into mouse